

Toxicology of Environmental Agents: A Blend of Applied and Basic Research

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The purpose of this paper is to present some of the more carefully studied aspects of the preclinical toxicology of antineoplastic agents, and apply these findings (with certain obvious cautions) to the area of environmental toxicology. Most of the studies to be described here are the result of applied toxicologic studies performed in support of the clinical introduction of new chemicals in the treatment of cancer. These considerations are somewhat superficial and restricted due to the fact that many questions regarding the chemical mechanisms of action, and the absorption, distribution, excretion, and metabolism of these drugs are unanswered. However, as valuable as this basic pharmacologic information will someday be to the complete understanding of therapeutic and toxic actions and effects, waiting is not a realistic option. The cancer problem is now. Thus, we are faced with the necessity of defining problems and taking reasonable actions based on the currently available data base; while, at the same time supporting, appreciating, and being careful to nurture more basic studies. The ideal situation is obviously a blend of applied and basic research. A similar situation most likely exists in the area of Environmental Toxicology.

Preclinical toxicology has been defined (1) as an area of Economic Toxicology primarily concerned with the development of drugs. Anticancer agents, almost by design, are extremely toxic and potent, nonspecific, and have a very low therapeutic index. The major toxic effects of immediate concern are usually acute or subacute effects, and because of the seriousness of the disease being treated, probable long-term toxic hazards are reluctantly accepted. The physicians using these drugs are well aware of their toxic potential, and clinical support facilities are excellent. Considering these aspects it appears that the experimental (preclinical) definition of the toxicity of anticancer drugs is probably the most straightforward of any clinically useful drug.

The toxicologist concerned with environmental agents has almost the opposite situation. Environmental toxicology is concerned primarily with the biological effects of chemicals that are encountered by man either incidentally because they are in the atmosphere, or by contact during occupational or recreational activities, or by ingestion of food additives. When air is the source of chemicals, it is obvious that exposure is unavoidable, whereas exposure to chemicals used in industry is determined by industrial practices. With the increasing rate of synthesis and the growing commercial use of chemicals, it seems that no one is entirely free of exposure to a variety of chemicals capable of producing undesirable effects on biologic tissues. The real and potential haz-

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ards of environmental chemicals are difficult to define, exposure levels are hard to quantitate, and acute toxicity is much less of a concern than are long term risks such as carcinogenesis and mutagenesis.

At the present time, almost half a million products are used industrially. Toxicity data are available on a few of these chemicals, and Gerarde (2) has presented a partial classification of such chemicals. Chemicals may be added to the feed of animals for therapeutic purposes or as pesticides. Each has the potential of becoming part of man's diet. Geiling and D'Aguanno (3) have listed some of the drugs that are commonly added to animal feed. The use of pesticides and insecticides in agriculture also presents the possibility of residues of these chemicals being present when the food is consumed. Chemical preservatives added to processed foods become contaminants when the food is consumed. The study of the limitations that must be observed in regard to food additives, and the evaluation of safety from harmful effects of such chemicals is also the responsibility of the environmental toxicologist.

The toxicologic study of any chemical involves essentially the same disciplines. These are presented in Figure 1, and represent the necessary expertise associated with any consideration of a potential health hazard present in the biosphere. Environmental toxicology might therefore be described as that branch of toxicology which deals with incidental exposure of biologic tissue, and more specifically in man; to the study of chemicals that are basically contaminants of his atmosphere, food, or water. This description includes the study of the causes, conditions, effects, mechanisms, and limits of safety of such exposure to chemicals.

Preclinical Toxicology of Anticancer Agents

The Laboratory of Toxicology of the Chemotherapy Program at the National Cancer Institute from which I recently transferred, is responsible for the preclinical toxicology of new anticancer agents and

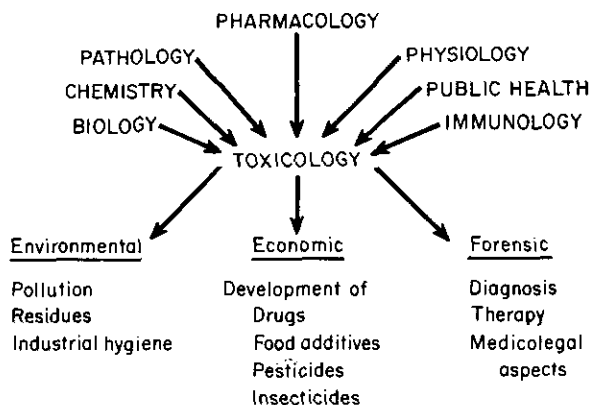


FIGURE 1. Disciplines involved in toxicity studies of chemicals.

investigates basic aspects of the pathogenesis of drug-induced disease. The structure and organization of the Chemotherapy Program has provided a large amount of carefully recorded experimental and clinical information dealing with the toxic effect of these drugs. These data provide an information base for a continuing evaluation of the effectiveness of these experimental toxicologic studies in warning the clinician of probable toxic effects in man. Special emphasis is placed on the qualitative (clinical signs; chemical, hematologic, and pathologic lesions) and quantitative (dose) predictiveness of laboratory animal for the human situation. I would like to present some of the successes and failures experienced recently. Hopefully, it will be of interest to all toxicologists and form the basis for further discussion to compare and contrast these findings regarding anticancer agents with toxicologic problems concerning environmental chemicals other than drugs.

The Laboratory functions to accomplish the following goals concerning the toxicity of new antineoplastic agents:

1. Establish defined levels of toxicity for each new agent using both dogs and monkeys.
2. Indicate the major organ toxicity in both experimental species.
3. Evaluate the predictability of observed toxicity.

4. Determine the reversibility of toxic effects.
5. Compare the consistency of qualitative and quantitative toxicity within and between experimental species.
6. Determine the influence of dosage schedules on toxicity.
7. Alert the clinician to the possibility of delayed drug toxicity.
8. Describe the etiologic aspects of drug-induced toxicity as indicated by hematologic, chemical, and histopathologic findings.
9. Suggest a dose for initial Phase I clinical trials.

In the past, confusion has surrounded the use of "MTD" to indicate both a minimally toxic dose and the maximum tolerated dose of a drug. MTD was defined by Freireich and his coworkers (4) as the highest dose killing none of the dogs or monkeys treated. At the same time, MTD was defined by the toxicologists as the dose which produced only minimal reversible toxicity, and this is the definition used by Schein *et al.* (5). To alleviate any further question regarding defined levels of toxicity which must form the basis for all quantitative comparison and extrapolation, the following definitions of levels of toxicity were established:

Highest Nontoxic Dose (HNTD): The highest dose at which no hematologic, chemical, clinical, or pathologic drug-induced alterations occurred; doubling this dose produces aforementioned alterations.

Toxic Dose - "Low" (TDL): The lowest dose to produce drug-induced alterations in hematologic, chemical, clinical, or pathologic parameters; doubling this dose produced *no* lethality.

Toxic Dose - "High" (TDH): The lowest dose to produce drug-induced alterations in hematologic, chemical, clinical, or pathologic parameters; doubling this dose produces lethality.

Lethal Dose (LD): The lowest dose to produce drug-induced death in any animals during the period of observation.

In addition to establishing the dose level

that produces the defined levels of toxicity for each schedule tested, a figure to indicate the slope of the dose-response is indicated. After discussing the relative value and problems of a mathematically derived slope, it was decided to use a ratio of the Lethal Dose to the Highest Nontoxic Dose in a manner similar to a therapeutic ratio. The most common ratio of the LD to the HNTD for anticancer agents is eight (drugs are tested by halving or doubling the initial dose). A lower number would indicate a steeper slope and a more rapid increase from nontoxic to toxic drug doses. A number larger than eight would indicate a more shallow slope and a more gradual increase from nontoxic to lethal levels with an accompanying increased margin of safety.

This approach using dose ratios can be questioned due to the fact that the ratio of two doses ignores the possibility of a non-linear dose response curve. However, past experience demonstrates that it usually takes four dose steps (1-2-4-8) to achieve the four levels of toxicity previously described which range from nontoxic to lethal. Any variant would be recognized quickly and further investigated.

Defined levels of toxicity for environmental chemicals would also be valuable. After a quick review of existing toxicity data for a variety of common environmental chemicals, a couple of points became apparent. It is difficult to find complete toxicologic studies on environmental chemicals in the literature. It also seems that all the chemicals reviewed are potent in that lethality can be produced although most are relatively much less potent than the anticancer agents. The threshold dose for toxicity (HNTD) varies greatly, but once the toxic threshold is reached the dosage increments between the HNTD and lethality are remarkably similar. These studies, of course, involve acute or subacute toxicity studies which probably explains the steep slope in going from threshold to lethal doses. The definition of toxic levels for long term hazards is a much more difficult matter, and requires first an agreement on whether a threshold

dose even exists.

Standardized protocols are used at the National Cancer Institute (NCI) to perform the routine preclinical toxicology. Special emphasis is placed on clinical signs, clinical chemistry, hematology, and the proper examination of organs and tissues for pathologic lesions. Such protocols insure consistency within and between research laboratories. Of course, no single scheme of operations can set a prior limit to the objectives of preclinical toxicologic studies, and it is obviously inappropriate to set rigid specifications or criteria which apply uniformly to all experimental drugs. Nevertheless, an attempt must be made to outline basic procedures which should elicit adequate and useful toxicologic data for a critical evaluation of the probable toxic risks involved during initial clinical trial or human exposure. These procedures must obviously be applied with judgment, and necessary modifications are introduced as indicated by preliminary findings. These protocols are available from NCI, and have been described previously (6).

The only points to make here in regard to Environmental Toxicology are self-evident: some standardization of toxicologic procedures is essential here if complete (as possible) studies are to be performed, information gathered from a number of different laboratories, results compared, and extrapolations made to humans. In this regard I would suggest that a generalized approach to environmental toxicology be considered while realizing that the situation with environmental chemicals and antineoplastic drugs are not entirely analogous (7). The general aim would also be to insure coordination and cooperation between the various federal and state agencies involved. In the toxicology area, such an approach would seek to insure that the more essential pharmacologic and toxicologic components necessary for any evaluation of a chemical are available. It is obviously rather early for such planning in the environmental health area as an optimal data base is not currently available. Therefore, great care must be

taken so that any structuring of general efforts or more specific toxicologic approaches in regard to environmental health does not in any way compromise the efforts of more basic scientists who are currently involved in building the essential data base, but whose projects might not fit into some niche of a "linear array". A broad outline of some important pharmacologic and toxicologic considerations is presented in Table 1, and I will refer to this in more detail later.

A great deal of effort has gone into the evaluation of the predictiveness of experimental studies in dogs and monkeys for toxicities in man. This area is being continually reviewed, and the recent paper by Schein and coworkers is an outstanding documentation of the effort (5). These workers documented the usefulness of dogs and monkeys in predicting potential qualitative drug toxicity in man by examining retrospectively twenty-five anticancer agents of diverse chemical and functional classification. It was found that the large animal screen served to alert the physician to a major proportion of the total spectrum of drug effects which were encountered during the clinical use of a new chemical compound. The dog and monkey correctly predicted bone marrow depression, gastrointestinal disturbance, and hepatotoxicity for each drug producing these effects in the clinic. In the case of renal, cardiovascular, and neuromuscular toxicity, however, the large animal screen failed in some instances to predict these toxicities in man. Unfortunately, the correct predictions were accomplished at the expense of a high percentage of false positives (animal positive; human negative) which resulted from the necessity of using severely toxic dose levels in order to demonstrate all potential toxicities inherent in any compound. Tables 2, 3, and 4 present data from this study which demonstrate the efficacy of the dog alone, monkey alone, and dog and monkey together as predictors of organ specific toxicity in man. It can be seen that the dog and monkey each predicts about the same percentage of true positives (animal positive; human posi-

Table 1. Important Aspects of A Toxicologic Study of A Chemical Agent

<p>I. Chemical and physical aspects.</p> <ul style="list-style-type: none"> a. Analyze purity of chemical and identify contaminants. b. Determine methods for extraction from biological material and subsequent quantitation. c. Synthesize necessary breakdown and/or metabolic products. d. Determine ionization constants and lipid solubility. e. Investigate structure-activity relationships. f. Analyze binding forces in chemical-protein (receptor) interaction. 	<ul style="list-style-type: none"> f. Consider chemicals or conditions which alter metabolism. g. Determine species differences and genetic variations.
<p>II. Absorption, distribution, and elimination.</p> <ul style="list-style-type: none"> a. Define routes of exposure (skin, lung, gastrointestinal tract, etc.) b. Determine apparent volumes of distribution. c. Assess binding of chemical to plasma and tissue proteins. d. Establish mechanisms of translocation and determine storage depots. e. Quantitate passage across biological membranes (CNS, intestine, placenta). f. Investigate routes of elimination (renal, biliary, lungs, etc.). g. Determine kinetics of uptake, distribution, and elimination for exposure by various routes. 	<p>IV. Routine acute and subacute toxicology.</p> <ul style="list-style-type: none"> a. Establish dose-response relationships for biological effects and toxicity. b. Determine LD-50's for various species using potential routes of exposure. c. Indicate potency, toxicity, cumulative effects, and margin of safety. d. Investigate the incidence of hyposensitivity and hypersensitivity. e. Determine general pharmacologic profile for chemical. f. Investigate activation and suppression of the immune response. g. Determine sensitizing potential and basis of chemical allergy. h. Establish antidotal systems for intoxication.
<p>III. Biotransformation.</p> <ul style="list-style-type: none"> a. Determine fate of chemical. b. Identify metabolites. c. Establish sites of metabolism. d. Indicate the chemical pathway of biotransformation. e. Establish the biological activity of metabolites. 	<p>V. Routine longterm toxicology</p> <ul style="list-style-type: none"> a. Determine functional lesions (sensory, cardiovascular, neuromuscular, etc.). <p>VI. Special longterm toxic hazards.</p> <ul style="list-style-type: none"> a. Actions on fertility and reproduction. b. Establish teratogenic potential. c. Investigate mutagenesis. d. Assess carcinogenic potential. <p>VII. Statistics.</p> <ul style="list-style-type: none"> a. Validity of experimental designs and conclusions. <p>VIII. Classification and analysis of toxic effects.</p> <p>IX. Current assessment of health hazard associated with chemical.</p>

tive), the monkey produces fewer false positives, but more false negatives (animal negative; human positive). A major problem

is that our current model systems predict the obvious, and often miss the unexpected toxicity. Anticancer drugs are known to

Table 2.—Dog as a Predictor for Organ Specific Toxicity in Man* (5)

Organ system	TP‡ (%)	FP§ (%)	TN (%)	FN¶ (%)	FN**	No. of compounds
					TP + FN (%)	
Injection site	16	36	40	8	33	25
Integument	12	32	40	16	57	25
Cardiovascular	28	24	36	12	30	25
Respiratory	16	64	16	4	20	25
Bone marrow	80	12	0	8	9	25
Lymphoid	4	72	24	0	0	25
Gastrointestinal	92	8	0	0	0	25
Liver	52	44	4	0	0	25
Renal	32	56	4	8	20	25
Neuromuscular	24	60	12	4	14	25

*TP = true positive; FP = false positive; TN = true negative; FN = false negative.

‡Toxicity observed in both dogs and man.

§Toxicity observed in dogs but not in man.

||No toxicity observed in dogs or man.

¶Toxicity not observed in dogs but recorded in man.

**Corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

Table 3.—Monkey as a Predictor for Organ-Specific Toxicity in man* (5)

Organ system	TP‡ (%)	FP§ (%)	TN (%)	FN¶ (%)	FN**	No. of compounds
					TP + FN (%)	
Injection site	13	26	52	9	40	23
Integument	13	17	57	13	50	23
Cardiovascular	22	26	30	22	50	23
Respiratory	13	48	30	9	40	23
Bone marrow	83	13	0	4	5	23
Lymphoid	0	31	65	4	100	23
Gastrointestinal	74	9	0	17	19	23
Liver	52	35	13	0	0	23
Renal	35	48	13	4	11	23
Neuromuscular	22	30	39	9	28	23

*TP = true positive; FP = false positive; TN = true positive; FN = false negative.

‡Toxicity observed in both monkeys and man.

§Toxicity observed in monkeys but not in man.

||No toxicity observed in monkeys and man.

¶Toxicity not observed in monkeys but recorded in man.

**Corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

Table 4. The Combination of Dog and Monkey as a Predictor for Organ-Specific Toxicity in Man* (5)

Organ system	TP‡ (%)	FP§ (%)	TN (%)	FN¶ (%)	FN**	No. of compounds
					TP + FN (%)	
Injection site	16	36	40	8	33	25
Integument	24	36	36	4	14	25
Cardiovascular	36	32	28	4	10	25
Respiratory	16	76	4	4	20	25
Bone marrow	88	12	0	0	0	25
Lymphoid	4	76	20	0	0	25
Gastrointestinal	92	8	0	0	0	25
Liver	52	48	0	0	0	25
Renal	36	56	4	4	10	25
Neuromuscular	24	60	12	4	14	25

*TP = true positive; FP = false positive; TN = true negative; FN = false negative.

‡Toxicity observed in both animals and man.

§Toxicity observed in animals but not in man.

||No toxicity observed in animals and man.

¶Toxicity not observed in animals but recorded in man.

**Corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

generally affect the bone marrow, lymphoid tissues, gastrointestinal tract and less commonly to exhibit hepatic and nephrotoxicity. Cardiovascular, pancreatic, central nervous and sensory lesions are much less common. The first group is almost always predicted; the second group is too often missed in the original preclinical study.

In regard to Environmental Toxicology, the major points of these qualitative evaluations are that the dog and monkey (and probably the rodent) reliably predict for many of the toxic effects of a chemical administered, either intentionally or inadvertently, to man. To achieve the best prediction, experimental dose levels must be escalated to lethality, and a price in false positives must be paid. In studying extremely high doses of environmental chemicals to determine their acute toxic potential, the distinction must be made between these dose levels and the concentrations present in the environment. However, the apparent discrepancies found between experimental doses of environmental chemicals needed to cause acute or subacute effects, and the chemical concentrations found in the environment should not discredit in any way

these basic studies. There is always the possibility that as more is learned concerning the shape of the dose-response curves, and the nature of receptors in experimental animals and man that some of the large discrepancies between doses and exposure levels for man which are so apparent today might not be real tomorrow. Nevertheless, generally accepted concepts of comparative dose-response relationships and toxic thresholds should restrain somewhat the over zealous reporter (or scientist) looking for headlines. Everyone agrees that better and more reliable experimental test systems must be sought from the ever expanding scientific base to enable specific prediction of the wide variety of possible toxicities for man associated with chemical exposure.

Most toxicities associated with anticancer drugs are reversible if detected early enough. This fact emphasizes the need for reliable clinical tests which will provide an early warning of drug-induced morphologic or functional changes. The obvious exceptions to the above statement regarding reversibility are immunologic sensitization and neurologic damage, and one should also add teratogenic, mutagenic and carcinogenic effects to

the list of nonreversible toxicities. In all cases, these nonreversible toxicities are of the greatest concern. Unfortunately this area too is hobbled by inadequate predictive test systems.

The relative doses of a chemical which produce toxicity or lethality must be continually re-evaluated. To assess these quantitative aspects of drug toxicity, the data compilation by Freireich et al. (4) is useful. Toxicity data for small animals (mouse, rat, and hamster), large animals (dogs and monkeys), and humans were gathered, placed on a reasonably similar basis, and compared quantitatively. Each animal species and all species combined were used to predict the toxic doses in man based on milligram per square meter of body surface area (mg/m^2). The experimental animal test systems used to evaluate the toxicities of potential anticancer drugs correlate remarkably well with the results in man. Figure 2 presents a representative comparison of toxicity data on anticancer agents for the mouse, rat, dog, monkey and man. One can see that when these data are expressed on a mg/m^2 basis there is rather good agreement between the doses which cause toxicity in experiment animals and man. However, the mg/m^2 is most useful when extrapolating toxicity data for small animals to larger animals or man. It appears to be much less useful when extrapolating toxicity data derived from dogs and monkeys to man. This is most likely due to the variability of the experimental data, and the relatively small conversion factors necessary to convert mg/kg doses in dogs and monkeys. The body surface area conversion factor is, of course, used in a number of areas in physiology and medicine, and has been discussed often in relation to drug dosage. The usefulness of this type of dosage extrapolation to the toxicity of environmental agents is largely unexplored due to the problems of demonstrating or quantitating toxicity in man.

Valuable lessons in regard to the effect of dosage schedules on therapeutics response and toxicity have been learned from the work of Skipper and coworkers and others

(8). The principal objective of schedule dependency antitumor testing in rodent tumor models is to obtain information on optimal conditions of drug use for designing toxicologic studies in large animals and, ultimately, for use by clinicians. Schedule-dependency studies may also furnish leads for uncovering biochemical sites of action. The increasing awareness of the relationship between drug effects and phases of the cell cycle suggests that animal studies relating dose schedule to the toxicology, pharmacology, and biochemical actions of the drug and to the growth kinetics of malignant and normal cells can establish important principles for improving clinical treatment. In addition to the kinetics of cell proliferation, the sites of drug action at the molecular level, and the maximum drug concentration (c) and the time (t) that an effective chemical level is maintained at the target site are also important. Lessons learned from experimental chemotherapy are valuable in toxicology.

Various treatment schedules are necessary to assess the toxicity of drugs which have demonstrated specific schedule sensitivity during experimental therapeutic tests with transplantable tumors. Following tumor inoculation, new drugs are routinely tested utilizing a variety of treatment schedules.

A comparison of the optimally effective dose (LD-10) and the increase in survival times for cytosine arabinoside (NSC 63878) is presented in Table 5. It is obvious that very low doses administered every three hours on days 1, 5, and 9 is the most effective schedule. This treatment regimen resulted in a four fold increase in life span, and 70% long-term survivors. Similar dramatic changes in toxicity are associated with various schedules, and some of these data are presented in Table 6. Total doses which result in lethality for ten percent of the mice are presented. It can be seen that treatment every three hours, essentially the optimal therapeutic schedule, is the most toxic. Unfortunately, the cell turnover time for the target tumor and normal tissues is not very different. The serum half-time for cytosine

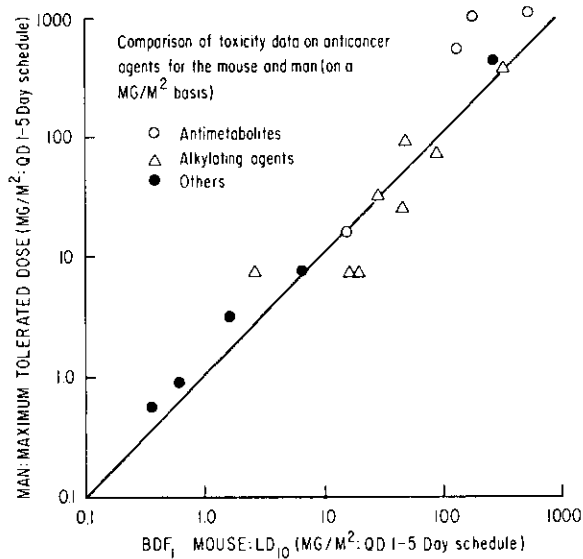
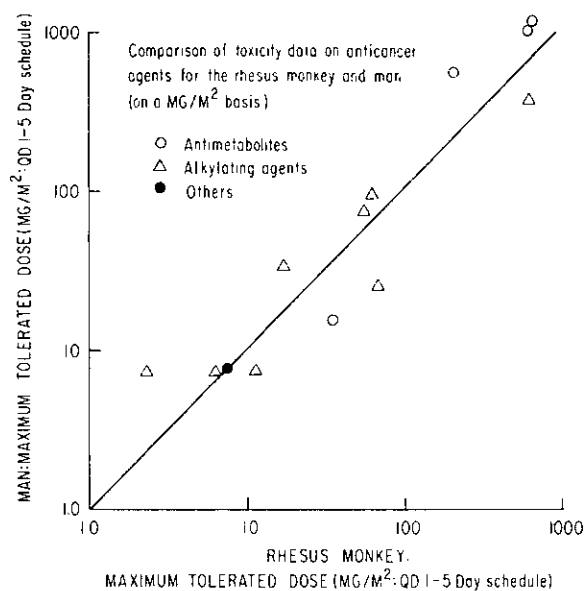
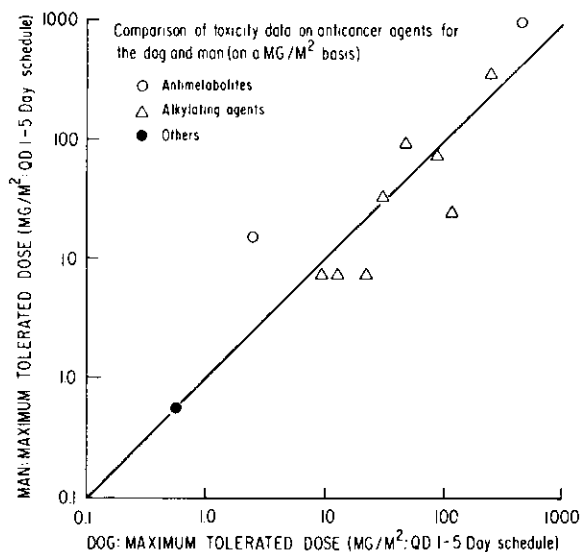
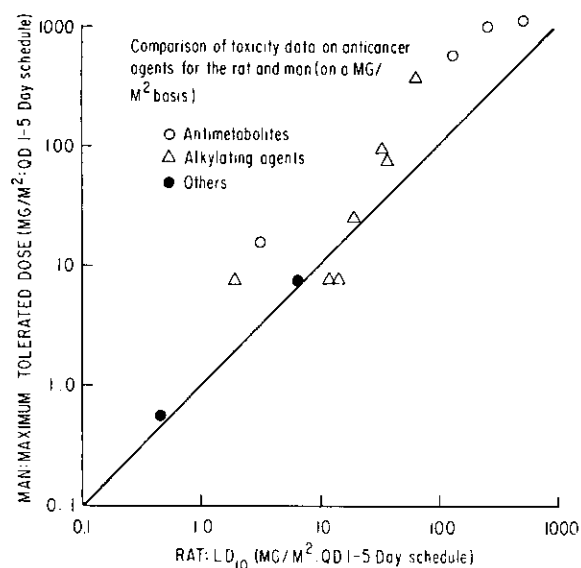


FIGURE 2. Comparison of toxicity data on anti-cancer agents for animals and man.

Table 5. Schedule Dependency Study with Cytosine Arabinoside (8)

Ip treatment schedule †	Optimal dose (mg/kg/injection)	ILS $\frac{\ddagger}{\%}$
Day 1 only	2312	33
q3h, Day 1 only	23	78
Days 1-9	39	100
Days 1, 5, 9	2313	128(1/10)
q3h, Days 1, 5, 9	14,23	>400(7/10)

† L1210 leukemia inoculated intraperitoneally on day 1 in BDF₁ mice.

‡ Increased life span; number of 45 day survivors shown in parentheses.

arabinoside is about 20 minutes (longer for very high doses), and the short term treatment dose needed to produce lethality is very high. With a drug like this, very high serum concentrations are tolerated for relatively short period of twelve hours or less if the treatment is not repeated too soon. If the treatment is continued for 24 hours or more, toxicity is much greater than cumulative when compared to exposures of 12 hours or less. Continued exposure of 24, 48, 72 and 96 hours provides almost cumulative

toxicity. Interestingly, treatment intervals of 48-96 hours allow for very significant tissue recovery in the mouse. Therefore, when the experimental antitumor activity indicates schedule dependency, the toxic effects of prolonged drug levels by either infusion or repeated injection are evaluated. These dramatic effects of treatment schedule on drug toxicity must also warn the environmental toxicologist to be alert to sometimes startling effects as a result of exposure time, especially in the toxicity areas dealing with effects of cell replication or differentiation.

Following acute or subacute drug treatment, observation periods of at least 45 days are necessary to insure an accurate evaluation of the anticancer agent's potential for delayed toxicity. The nitrosourea anticancer agents produce life threatening toxicity many weeks after a single treatment in both laboratory animals and man. Delayed organ toxicity in preclinical studies must always alert the physician to this clinical possibility. However, the clinical delayed toxicity might be associated with a different organ system than that observed in dogs or monkeys.

Delayed effects of acute or subacute ex-

Table 6. Toxicity of Cytosine Arabinoside to the BDF₁ Mouse (on a Total Dose Basis) When Administered According to Different Schedules (8)

Schedule	Approx. Period of R _x (hrs)	Interval Between Doses (hrs)	Total Dose	
			mg/m ²	End Point
Single dose	Single dose		9,000	LD ₁₀
q3hrs (x 3)	6	3	11,000	LD ₁₀
q2.5hrs (x 4)	7.5	2.5	>3,000	LD ₁₀
q3hrs (x 8)	24	3	560	LD ₁₀
q3hrs (x 16)	48	3	700	LD ₁₀
q3hrs (x 24)	72	3	750	LD ₁₀
q3hrs (x 32)	96	3	800	LD ₁₀
qd (x 6)	120	24	3,240	LD ₁₀
qd (x 15)	340	24	1,500	LD ₁₀
q2d (x 8)	340	48	15,000	LD ₁₀
q4d (x 4)	300	96	21,000	LD ₁₀

posure to environmental chemicals, although not exactly analogous, are probably the problem that needs most serious consideration. Teratogenic, mutagenic, and carcinogenic toxicities fit this definition. Of major concern recently, have been reports of vaginal cancer in young girls years after their mother was exposed to diethylstilbesterol during pregnancy. This is a unique example of delayed toxicity.

To be most effective, reports of toxicology must move away from purely descriptive accounts to a careful evaluation of the hematologic, chemical, and histopathologic findings in a best attempt to elucidate the genesis and subsequent development of the toxic state using available test methods. In other words, toxicology must utilize the results from a number of static parameters to start to resolve the complexities and obscurities of the drug-induced disease. The ultimate goal of the toxicologist must be an understanding of the total mechanism of toxic effect. A more complete knowledge of mechanisms would hopefully allow for an early prediction of subsequent physiologic and pathologic alterations. To a degree, the study of anticancer agents has partially reached this point. Especially with alkylating agents and antimetabolites, the strong, but still incomplete, information base allows one to predict fairly accurately the toxic effects of most new drugs. Almost all of the unexpected toxicologic problems with new anticancer agents are associated with new categories of drugs (enzymes, heavy metals, etc.) that have yet to achieve the same degree of appreciation.

Obviously, it takes time to develop a base of understanding concerning the mechanisms of toxicity of a chemical; and that it is a continuing process. This must also be the goal of Environmental Toxicology, and the descriptive information must be gathered in a complete manner to facilitate the correlation of biochemical and functional effects.

The detailed preclinical and clinical studies of anticancer drugs have facilitated the continuing evaluation of the predictiveness of preclinical investigations. I have

already mentioned important studies of the quantitative and qualitative correlation between experimental animals and man (4,5). The actual extrapolation of these data to man for an untested chemical is one important test of these experimental studies. However, the scientific value of experimental studies does not rise or fall on the ability of predictions made today from experimental animals to be immediately verified by the present clinical testing techniques. Such a verdict would overlook the many problems which still exist in understanding interspecies differences in pharmacokinetics. Often with better and more complete information, previous poor prediction will be explained in terms of the accessibility of a chemical to a particular receptor. The final evaluation of predictiveness must always await a comparison of comparable chemical concentrations reaching comparable receptors.

The Laboratory of Toxicology has recently carefully examined the qualitative relationships between toxic doses of antitumor agents in large animals and man (6). We have extended the data of Freireich *et al.* (4) and Schein *et al.* (5) by using essentially a probit transformation to present the cumulative distribution of antineoplastic drugs when the ratio of toxic doses in dogs and/or monkeys are compared to man. The individual toxic dose ratios were considered to be members of a normalized cumulative distribution. Any drug's dose ratio can then be associated with a cumulative fraction of the total sample of ratios. For instance, half of the ratios would be less than 50% and half greater. The cumulative fraction may be considered numerically equivalent to the probability that a given dose will exceed the clinical tolerated dose and thus provide an estimate of the clinical risk of an untried clinical drug candidate based on the animal data and the pattern of relationships of similar drugs. Thus, the cumulative fraction is plotted against the ratio of the clinic dose to the animal toxic dose, and each point represents a different anticancer agent. If the mean ratio of clinical toxic dose to animal toxic dose was 1 (perfect predictability) then, depend-

ing on variation, one would expect half of the drugs to be less toxic to humans and half to be more toxic based on the animal data. A representative plot of this type of data is presented in Figure 3.

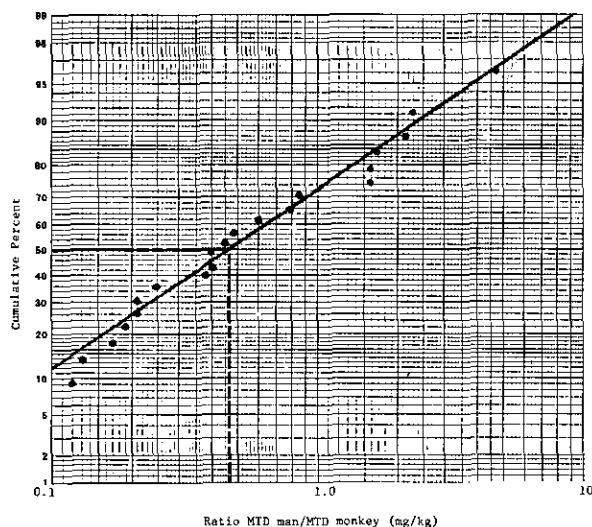


FIGURE 3. Cumulative distribution of clinical MTD: animal MTD ratios of antitumor drugs (6).

Using this system of analysis, quantitative comparisons can be made between experimental species and man; and the most effective expression of dose (mg/kg or mg/m²) can be determined. A point of particular interest is the probability of exceeding a dose ratio of 0.1, or 1/10 of the toxic dose in the most sensitive experimental animal species. This derivation is commonly used to establish the initial clinical doses for a new drug. A more recent innovation is the proposal that initial clinical doses be derived

as one-third of the animal tolerated dose based on mg/m². These data are presented in Table 7. A dose one-tenth that of the dog mg/kg tolerated dose carries a risk of 4% of exceeding the clinical tolerated dose; the corresponding risk associated with monkey data is 9%. When one-third of the dose on a mg/m² basis is used, this type of analysis indicates an equivalent risk associated with monkey and dog data of about 10%; about double that of using one-tenth the mg/kg tolerated dose and ten times that of using one-tenth of the mg/m² dose. These studies allow one to determine statistically the toxic risk associated with any extrapolation from experimental animal data in the selection of the initial human dose. There is no question that some level of risk must be accepted in the selection of the initial clinical dose in order to insure that large numbers of seriously ill patients do not receive ineffective drug levels. On the other hand, the dose must not be so high that the patient is subjected to serious toxic hazards. Therefore, two points are clear; 1) a reasonable level of clinical risk must be defined and accepted by the clinician, and 2) it must be recognized that statistically with any risk level selected, eventually a certain percentage of drugs will be introduced into the clinic at doses which will produce toxicity. The difficult question remains—what level of risk is acceptable?

Some level of risk must also be accepted in regard to environmental chemicals based on the benefits of the chemical to man. Obviously, the acceptable risk level must be many logs lower than that associated with

Table 7. Clinical Risk in Extrapolating Dog and Monkey Data to Man (6)

Predicting species	Dosage units	Regression mean ratio (MTD clinical/MTD preclinical)	Clinical risk at	
			1/10 MTD	1/3 MTD
Dog	mg/kg	0.86	3%	
	mg/m ²	1.5		7%
Monkey	mg/kg	0.5	12%	
	mg/m ²	1.4		11%

the introduction of a new anticancer agent. However, the problems are the same in each case. That is, reliable model test systems must be produced, toxic effects demonstrated, and extrapolation made from experimental species to man. At that point, the same question remains—what level of risk is acceptable?

A Blend of Applied and Basic Research

As mentioned previously, the evaluation of the toxic effects of environmental chemicals presents special challenges. Toxic potentials are difficult to define, exposure levels are hard to quantitate, exposure is unintentional, readily apparent toxic effects in humans are rare, and acute toxicity is much less of a concern than are long term risks such as carcinogenesis and mutagenesis. The lack of a carefully directed overall approach to environmental toxicology has produced too little solid information, and too many data from experiments inadequately designed to yield the maximum benefit. I feel the scientific predictive base for environmental toxicology needs strengthening in both approach and methodology to enable one to use animal studies to predict potential toxicologic problems and assess their risk to human health.

With so many serious problems involving drugs and other chemicals affecting the health of living organisms, a way must also be found to attract the best trained investigators into applying their special talents to a study of the broad, basic underlying mechanisms of chemical toxicity. The newly graduated Ph.D. faces a dilemma. Throughout the years of graduate training, the chastity and purity of basic science are emphasized. The subject of his dissertation is usually very narrow, suggested by an advisor as an extension of his own laboratory interests, and requires years of in-depth study. He often inherits experimental tools and methods that are well developed and understood, and the application of the problem is secondary. His graduate training experience leads the new graduate to seek a

first position where he can apply familiar methods to extend the observations made during his dissertation work. Thus, too many scientists select some very narrow aspect of an overall toxicologic problem, and pursue it without enough concern for the total picture. Studies often represent the application of analytical or biological test methods already operating in a laboratory, into which are randomly fitted a series of environmental chemicals. These bits and pieces of information obviously represented good intentions and a substantial effort in time and resources. However, very important basic toxicologic aspects are often unexplored. The problem is lack of coordination. It is impossible to construct a clear picture of the hazard associated with many environmental chemicals. It is as if the pieces of a puzzle are fitted together at random rather than being interlocking parts of a potentially serious problem.

The problem of basic vs. applied research must be resolved. I share with the basic researcher the fear that things could move to the point where some research czar would direct the selection of the research area and detail of the experimental approach. However, some attempt to coordinate (not dictate) areas of research should not be rejected as quickly.

The recent Conference on Polychlorinated Biphenyls (PCBs) (9), and the current review of the potential hazards to human health of nitrilotriacetate (NTA) (10), demonstrate the incomplete toxicologic picture available when one attempts to review the available literature in an attempt to establish the risk or hazards associated with chemicals already in the environment. Dr. Nelson summarized the PCB conference and presented a series of items urgently needing further clarification (11). These points included the areas of environmental transport, distribution, occurrence, and alteration. In addition he called attention to needed studies to clarify issues of comparative metabolic patterns by species which might account for some of the species variability in regard to toxicity. In regard to the mechanism of toxic actions Dr. Nelson

suggests, "Rather than to hastily examine the effects of PCB's on a myriad of in vitro test systems, it would seem wiser that these studies be carefully planned as aids to understanding the observed course of PCB poisoning in the intact animal". Put another way, there is an immediate need for a coordinated approach to environmental toxicity starting most often with the whole animal, and then moving to the test tube to insure that pieces of the problems are produced which help most to resolve them.

Table 1 lists broadly some of the more obvious areas of investigation for any chemical released to the environment. Questions concerning chemical and physical aspects; absorption, distribution and elimination; biotransformation; routine acute and subacute toxicology; routine long term toxicology; special long term hazards; statistics; and the classification and analysis of toxic effects are indicated. The surprising thing is how often concern is associated with an environmental chemical that has been randomly studied for years without producing even half of this basic information. My point is only this, each of these areas first requires the establishment of the general information which then should be extended by more in-depth studies to resolve basic mechanisms of action. I think this "first-step first" approach will also hasten the development of the information base necessary to produce better predictive systems.

Conclusions

The structured approach to the experimental and clinical study of anticancer agents has produced a background of carefully gathered information which is available for analysis. Questions regarding the effectiveness of experimental animal studies to predict chemically induced toxicity in man can be asked and partially answered. This organized program has not seriously compromised basic studies, but has brought the "pure" basic scientist closer to his fellow scientist charged with facing the more immediate problems. There is obviously a need

for both undertakings. Environmental Toxicology is also in need of a coordinating program to assist the gathering of information concerning the effects of chemicals on human health. The main goals of such a structuring would be greater completeness for the toxicologic information gathering process, and would provide "whole animal" observations which would be extended by more basic studies of mechanisms. This approach should speed the development of reliable predictive test systems, and present a much better toxicologic picture for the evaluation of biologic hazards associated with environmental chemicals.

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